REMARKS

The Examiner's attention is directed to page 11 of the Preliminary Amendment filed on March 24, 2005. Attached hereto is an executed Rule 132 Declaration showing that the substance of the present invention is effective to inhibit germination of spores, but does not inhibit the growth of vegetative cells.

The Experimental Procedures are discussed on pages 3-6 of the Declaration. The results are shown in Tables 1 and 2 on page 6 of the Declaration, which are reproduced below for the Examiner's convenience.

Table 1: Effect of the antibacterial substance on the inhibition of germination of spores

	Control	Antibacterial substance		
		1%	10%	30%
Number of colonies	1293	688	607	312
Inhibition rate		47%	53%	76%

Table 2: Effect of the antibacterial substance on the inhibition of growth of vegetative cells

	Control	Antibacterial substance		
		1%	10%	30%
Number of colonies	1333	1665	1553	1521
Inhibition rate		N/A	N/A	N/A

The Examiner should note that the antibacterial substance of the present invention inhibited germination of spores, but did not inhibit growth of vegetative cells. This effect is entirely different from the prior art antibacterial substances, wherein growth of vegetative cells is inhibited if the antibacterial substances inhibit germination of spores. Clearly, the effect of the present invention is not taught, suggested or obvious over the teachings of the prior art.

Favorable action on the merits is respectfully requested.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned to conduct an interview in an effort to expedite prosecution in connection

with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By 

Marc S. Weiner, #32,181
P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

MSW/sh
0397-0441P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	SAKAI, Takuo	Conf.:	7643
Appl. No.:	10/069,182	Group:	1651
Filed:	May 22, 2002	Examiner:	Ware, Deborah K.
For:	PROCESS FOR PRODUCING PLANT-ORIGIN ANTIBACTERIAL SUBSTANCE		

DECLARATION UNDER 37 CFR §1.132

Assistant Commissioner for Patents

Washington, DC 20231

Sir:

I, Mr. Takuo SAKAI, declare the following:

I am the inventor of the invention described in the above-identified application. I am fully knowledgeable of the disclosure of the above-identified application and the field of art of the present invention. I have read and understand the Advisory Action dated December 27, 2004 and the Interview Summary dated February 3, 2005 and the references cited therein; WO 01/07135 (WO'135), U.S. Patent No. 6,063,382 (US '382), EP 0880894 (EP '894) and Sakai et al. (Agric. Biol. Chem., Vol. 54, No. 4, pages 879-889, 1990).

A) 1. I am a citizen of Japan residing at Sakai-shi, Osaka, JAPAN;

2. (a) I received a doctor's degree in 1965 from the Graduate School of Agriculture, Kyoto University, Japan;

(b) I was employed by TANABE SEIYAKU CO., LTD. in 1965, where as a research worker in 1965-1970, I was involved in research work on production of functional saccharides (fructose 1,6-bisphosphate) and research work on production of coenzyme type nucleotide.

(c) From 1970 to 1990, I served as associate professor in the Faculty of Agriculture, Osaka University, Japan, where I was involved in research work on utilization of ligneous particles for the food circulatory system, research work on biological wrought of cotton fiber, research work on ume vinegar and research work on protopectinase enzyme. Papers about my research work on protopectinase enzyme were published in the Journal of Agricultural and Biological Chemistry in 1988, 1989, 1990, etc.

(d) From 1997 to 2003, I served as professor in the Faculty of Agriculture, Kinki University, Japan.

B) The present invention relates to a process of producing an antibacterial substance derived from a plant which includes disintegrating at least a part of the tissue of the plant and releasing the antibacterial substance therefrom, and bactericidal or bacteriostatic compositions containing as an active ingredient the antibacterial substance obtained by the process. The uniqueness of the present invention lies in the fact that the antibacterial substance is obtained from the tissue of the plant by disintegrating the plant tissue with an enzyme (e.g., protopectinase) capable of acting on protopectin to release a pectin substance and that the

antibacterial substance inhibits germination of spores from spore-forming bacteria and koji mold.

The following experiments were performed by me or under my direct supervision. It is clear from the data obtained from these experiments that the means of disintegrating plant tissue has a profound effect on the inhibition of germination of spores of spore-forming bacteria of the resulting composition.

The below experimental data shows that the antibacterial substance inhibits germination of spores but does not inhibit growth of vegetative cells.

An experiment is carried out as follows:

Material: Onion.

Bacteria examined: *Bacillus subtilis* ATCC 6633.

Method for preparing the antibacterial substance of the present invention:

An onion (a subterranean stem), 100 g (wet weight), was chopped into 0.5 to 1 cm squares and suspended in 100 mL of distilled water. Protopectinase from Trichosporon penicillatum (1600 IU/mL; the enzyme activity was determined in accordance with Sakai's method in Methods in Enzymology, Academic Press, vol.161, pp. 335-350) is added thereto, followed by stirring at 37°C for 3 hours. The resulting solution is centrifuged to remove the solid, thereby giving a supernatant liquid. The supernatant liquid is further purified by solvent extraction to give the antibacterial substance of the present

invention.

Method for measurement of the activity for inhibiting germination of spores:

Spores of Bacillus subtilis ATCC 6633 (Eiken Kagaku Kabushiki Kaisha) are suspended in saline to obtain the concentration of 2 x 10^8 spores/mL. The spore suspension is boiled for 10 minutes and immediately cooled on ice to kill the vegetative cells contained therein.

Thus obtained spore sample is diluted with saline to obtain the concentration of 10^8 spores/mL. 100 μL of the diluted spore solution and 10 μL (1%), 100 μL (10%), or 300 μL (30%) of the antibacterial substance of the present invention obtained above are added to 890 μL (1%), 800 μL (10%), or 600 μL (30%), respectively, of GYP medium (glucose 2%, yeast extract 0.3%, peptone 0.3%, pH 6.5). The mixture is incubated for 1 hour at the room temperature, then 100 μL of the mixture is spread onto GYP medium agar plate. The plate is incubated at 37°C for 24 hours and the number of colonies (vegetative cells germinated from spores) is counted.

As a control, distilled water is used instead of the antibacterial substance of the present invention.

The rate of inhibition of germination of spores is calculated by the following equation:

Inhibition rate (%) = (A - B) / A x 100

wherein A is a number of colonies observed on the control plate; and

B is a number of colonies observed on the plate where spores are treated with the antibacterial substance.

Method for measurement of the activity for inhibiting growth of vegetative cells:

Bacillus subtilis ATCC 6633 are inoculated into 1 mL of GYP medium and incubated with vigorous shaking at 37°C for 12 hours. Thus obtained pre-culture are inoculated to 100 mL of GYP medium in a flask and the flask is incubated with vigorous shaking at 37°C to attain the concentration of 2×10^8 cells/ml.

Thus obtained vegetative cell sample is diluted with saline to obtain the concentration of 10^8 spores/mL. 100 μL of the diluted cell solution and 10 μL (1%), 100 μL (10%), or 300 μL (30%) of the antibacterial substance of the present invention obtained above are added to 890 μL (1%), 800 μL (10%), or 600 μL (30%), respectively, of GYP medium (glucose 2%, yeast extract 0.3%, peptone 0.3%, pH 6.5). The mixture is incubated for 1 hour at the room temperature, then 100 μL of the mixture is spread onto GYP medium agar plate. The plate is incubated at 37°C for 24 hours and the number of colonies (vegetative cells germinated from spores) is counted.

As a control, distilled water is used instead of the antibacterial substance of the present invention.

The rate of inhibition of growth of vegetative cells is calculated by the following equation:

Inhibition rate (%) = (A - B) / A x 100

wherein A is a number of colonies observed on the control plate; and B is a number of colonies observed on the plate where cells are treated with the antibacterial substance.

Results:

The results are shown in the following Table 1 and Table 2.

Table 1: Effect of the antibacterial substance on the inhibition of germination of spores

	Control	Antibacterial substance		
		1%	10%	30%
Number of colonies	1293	688	607	312
Inhibition rate		47%	53%	76%

Table 2: Effect of the antibacterial substance on the inhibition of growth of vegetative cells

	Control	Antibacterial substance		
		1%	10%	30%
Number of colonies	1333	1665	1553	1521
Inhibition rate		N/A	N/A	N/A

From the fact that the antibacterial substance of the present invention inhibited germination of spores, but did not inhibit growth of vegetative cells, I firmly believe the inhibition effect of the antibacterial substance of the present invention is different from

that of antibacterial substances of the prior art which inhibit growth of vegetative cells if they inhibit germination of spores. Furthermore, I firmly believe that the skilled artisan would not find the inventive process as defined in claim 1 nor the product obtained from the inventive process as defined in claim 7, as presently amended, obvious over the cited prior art.

C) The undersigned hereby declares that all statements made herein based upon knowledge are true, and that all statements made based upon information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

05/3/29
Date

By _____
Signature

Takuo SAKAI
Typed Name